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Detection of *Mycobacterium tuberculosis* in a Reference Facility in Rivers State Using Three Laboratory Techniques

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ABSTRACT

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains a major public health challenge in Nigeria, particularly with the emergence of drug-resistant strains. Rapid and accurate laboratory diagnosis is critical for effective disease control and patient management. This study aimed to evaluate Ziehl-Neelsen (ZN) smear microscopy, GeneXpert Ultra assay, and culture on Lowenstein-Jensen (LJ) medium for the detection of *M. tuberculosis*. A total of 150 sputum specimens were collected from presumptive TB patients between July 2024 and May 2025 across six states in the South-South geopolitical zone of Nigeria. All samples were processed using standard biosafety measures and laboratory protocols in a reference laboratory in Rivers State. The overall TB prevalence based on acid-fast bacilli detection was 69.3%. The GeneXpert MTB Ultra assay detected *M. tuberculosis* in 89.3% of samples. Culture on LJ medium yielded growth in 47.3% of samples, with NTM detection rate of 6.8% and a contamination rate of 8.5%. Genexpert MTB Ultra demonstrated a high positivity of 80.7% almost similar to the results provided by the Zeihl-Neelsen. LJ culture, had significant issues with contamination (8.5%), resulting in a 47.3% positivity rate. The findings highlight the complementary roles of microscopy, molecular, and culture-based diagnostic techniques in TB detection. Integrating rapid molecular diagnostics with conventional methods is essential to improve diagnostic accuracy and strengthen TB control efforts in high-burden settings.

Keywords: *Mycobacterium tuberculosis*, Diagnosis, GeneXpert MTB Ultra, Lowenstein-Jensen culture, drug resistance.

Introduction

Tuberculosis (TB) remains one of the leading causes of mortality worldwide, particularly in sub-Saharan Africa. In Nigeria, TB continues to contribute significantly to morbidity and mortality, with mortality rates estimated to range between 150,000 and 200,000 deaths annually (World Health Organization [WHO], 2023). Globally, TB is associated with an estimated 10.6 million new cases and approximately 1.5 million deaths each year, making it one of the top infectious disease killers (WHO, 2023).

Nigeria is consistently ranked among the top 10 high TB burden countries globally, accounting for a substantial proportion of cases in Africa (WHO, 2023; Federal Ministry of Health [FMoH], 2022).

The burden of tuberculosis in Nigeria is further exacerbated by factors such as poverty, overcrowding, limited healthcare access, and the increasing prevalence of drug-resistant TB strains.

Access to prompt and accurate diagnosis, followed by timely treatment, has been identified as a critical factor in reducing TB transmission and mortality (Pai et al., 2015). However, diagnostic gaps remain a major challenge. A recent report by Gande et al. (2025) indicates that Nigeria accounts for approximately 6.2% of the 3.1 million "missing" TB cases globally, cases that are either undiagnosed or unreported.

Several diagnostic methods are available for TB detection, including microscopy, culture, and molecular techniques (WHO, 2023).

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Microscopy, particularly Ziehl-Neelsen (ZN) smear microscopy, remains the most widely used diagnostic method in resource-limited settings due to its simplicity, low cost, and rapid turnaround time. It enables the detection of acid-fast bacilli (AFB) in sputum samples, making it useful for initial screening. However, microscopy has limited sensitivity, especially in patients with low bacterial load, and cannot differentiate between drug-sensitive and drug-resistant strains (Steingart et al., 2006; Arora & Dhanashree, 2020).

Culture remains the reference standard for TB diagnosis due to its high specificity, sensitivity and ability to support drug susceptibility testing. However, the major limitation of culture methods, particularly on Lowenstein-Jensen (LJ) medium, is the prolonged turnaround time, typically requiring 4–8 weeks to yield results (Forbes et al., 2018). This delay can hinder timely clinical decision-making and disease control efforts.

In recent years, newer diagnostic approaches such as the GeneXpert MTB Ultra assay and line probe assays (LPAs) have been introduced for TB detection (WHO, 2023). These are molecular-based tests that detect the genetic material of *Mycobacterium tuberculosis* and identify drug resistance. Advantages of these methods include rapid turnaround time (within 2 hours for GeneXpert) and simultaneous detection of rifampicin resistance, which is a key marker for multidrug-resistant TB (Boehme et al., 2011).

Despite these benefits, their limitations include high cost, dependence on stable electricity and technical expertise, and reduced accessibility in rural or resource-constrained settings (Pai et al., 2015).

Considering the increasing adoption of non-traditional diagnostic techniques alongside conventional microscopy and culture, there is a need for continuous evaluation of these methods to determine their comparative diagnostic performance. Several studies in Nigeria and similar high-burden settings have assessed the effectiveness of different tuberculosis diagnostic approaches, particularly comparing microscopy, culture, and molecular techniques. For example, a study in Nigeria by Ejeh et al. (2018) demonstrated that the GeneXpert MTB Ultra assay showed significantly higher sensitivity compared to Ziehl-Neelsen microscopy, particularly in detecting cases with low bacillary load, while microscopy remained useful for rapid screening in resource-limited settingsand for follow-up testing. Similarly, comparative evaluations across multiple settings have consistently shown that GeneXpert provides superior diagnostic accuracy and rapid detection when compared with smear microscopy, with sensitivity values exceeding 90% in some studies (Boehme et al., 2011; Chandrik Babu et al., 2025).

In addition, studies comparing GeneXpert with culture methods have reported that while culture remains the reference standard due to its high sensitivity and ability to support drug susceptibility testing, its prolonged turnaround time significantly limits its clinical utility (Nadeem et al., 2022). Molecular methods such as GeneXpert, on the other hand, enable rapid detection of *Mycobacterium tuberculosis* and rifampicin resistance within hours, thereby improving early case detection and treatment initiation (Boehme et al., 2011).

Furthermore, newer comparative studies incorporating additional techniques such as loop-mediated isothermal amplification (LAMP) have reaffirmed that GeneXpert offers a balanced combination of sensitivity and specificity compared to microscopy and other rapid tests (Kumar et al., 2025).

Despite these advances, findings across studies have not been entirely consistent. Variations in diagnostic performance may be attributed to differences in laboratory infrastructure, technical expertise, specimen handling, and geographical location.

Importantly, there remains a relative paucity of such comparative diagnostic studies conducted in Rivers State, particularly in Port Harcourt. While some studies have evaluated tuberculosis control and diagnostic capacity in Nigeria broadly, limited data exist on the direct comparison of diagnostic techniques within this specific region. This highlights a critical gap in localized evidence necessary for optimizing diagnostic strategies in Rivers State.

This study, therefore, aimed to assess the detection of *Mycobacterium tuberculosis* in a reference laboratory in Rivers State, Nigeria using Ziehl-Neelsen microscopy, GeneXpert MTB Ultra assay, and culture on Lowenstein-Jensen medium.

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Materials and Methods

Study Design/Area/Population

This study was a cross-sectional, laboratory-based investigation conducted at a Reference Laboratory in Rivers State, Nigeria. The study population was drawn from the South-South geopolitical zone, comprising Rivers, Akwa Ibom, Edo, Cross River, Delta, and Bayelsa States.

A total of 150 sputum samples were collected from presumptive tuberculosis (TB) patients between July 2024 and May 2025, with 25 samples obtained from each of the six states. Eligible participants included consenting adults aged 18–75 years who were currently receiving anti-tuberculosis treatment.

Sample Collection and Processing

Early-morning sputum specimens were collected in sterile 50 ml leak-proof containers to ensure sample quality. All samples were transported to the laboratory using a standard triple-packaging system within ice-cooled transport boxes, thereby preserving specimen integrity and ensuring compliance with biosafety protocols.

All sputum specimens were analyzed for the presence of *Mycobacterium tuberculosis* using a combination of microscopy, culture, phenotypic and molecular methods (Otokunefor et al., 2018).

Laboratory Diagnosis

Ziehl-Neelsen (ZN) Staining

Sputum smears were prepared, air-dried, heat-fixed, and stained using the standard Ziehl-Neelsen technique (WHO, 2024). Slides were examined under oil immersion (1000× magnification) for acid-fast bacilli (AFB). Results were reported according to WHO grading guidelines.

Culture

Samples were decontaminated using the N-acetyl-L-cysteine–sodium hydroxide (NALC-NaOH) method and Phosphate Buffer Saline (PBS). Sediments resuspended in PBS were inoculated onto Lowenstein-Jensen (LJ) Medium and incubated at 37°C for up to 10 weeks. Cultures were examined weekly for growth. The presence of one or more colonies was considered positive (WHO, 2024).

GeneXpert Ultra Assay

Processed sputum samples were mixed with GeneXpert sample reagent and loaded into GeneXpert cartridges. The assay was performed according to the manufacturer's instructions, with results automatically generated within two hours, indicating TB detection status and rifampicin resistance (WHO, 2024; Cepheid, 2024).

Data Analysis

Data obtained from the study were analysed using descriptive statistics, including frequencies and percentages. The results were presented in charts to facilitate clear comparison of the diagnostic methods.

Results

Demographic characteristics of the study population revealed that more males were enrolled (58.7%, 88/150) with each of the six states contributing 25 samples, ensuring equal representation across the South-South region. Majority of participants were in the 21–30 years age group (Table 1).

Table 1: Patient Demographic Data

Variables	Number (%)
Sex	
Male	88 (58.7)
Female	62 (41.3)
Age Group	
≤ 20	18 (12.0)
21 - 30	44 (29.3)
31 - 40	39 (26.0)
41 - 50	26 (7.3)
51 – 60	18 (12.0)
≥ 61	5 (3.3)

Prevalence of tuberculosis in the study population showed that out of 150 sputum samples analysed, 89.3% (134/150) were positive for TB based on the results of all test methods. However, detection rates varied, with the highest positivity recorded using Gene Xpert Ultra (Figure 1).

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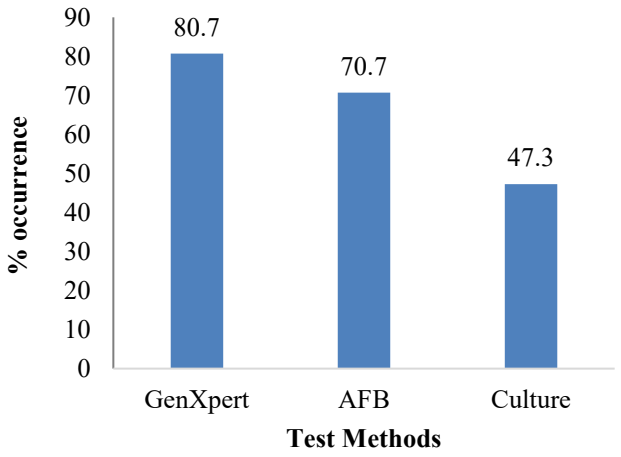


Figure 1: Prevalence of Tuberculosis (TB) from July 2024 – May 2025 using three test methods

The lowest rate of detection was observed following culture. In this case though no growth was observed only in 31 (20.7%) of cases with contamination occurring in 13 (8.5%). A similar level of TB detection was made across most of the age-ranges (Figure 2) apart from the 51 – 60 years range which was associated with the lowest detection rate. Similarly, the detection rate in states was similar ranging from 80% to 100% (Figure 3).

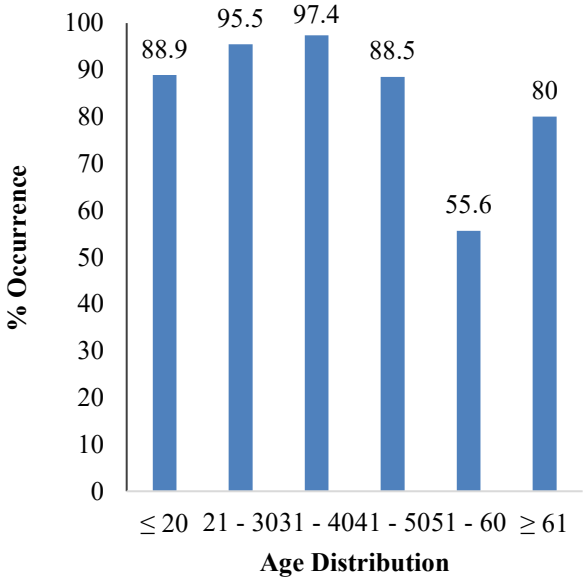


Figure 2: Occurrence distribution of TB detection based on age range

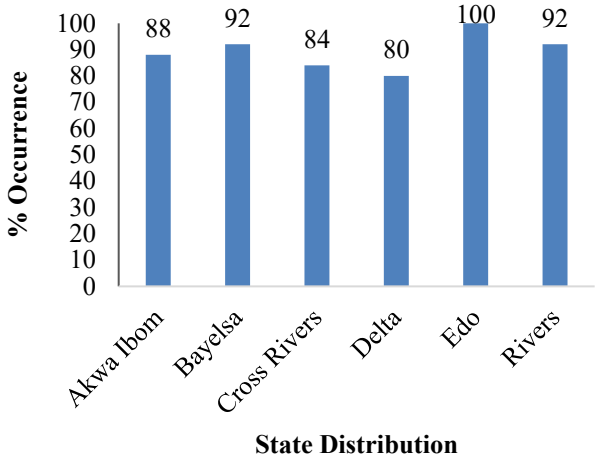


Figure 3: Occurrence distribution of TB detection based on State

Discussion

The global fight against tuberculosis (TB) remains urgent, with the 2024 TB report estimating 10.8 million cases worldwide, including 8.2 million new diagnoses, and more than one million deaths attributed to the disease. Among the most affected countries, Nigeria remains in the top 10 on all three high-burden lists: TB, HIV-associated TB, and MDR-TB (Adepoju et al., 2025). This study highlights the persistently high burden of tuberculosis (TB) in the South-South region of Nigeria, with an overall prevalence of 89.3%. This finding is comparable to reports from other Nigerian studies that documented very high TB positivity rates among presumptive TB patients. For example, Mohammad et al. (2024) reported a TB positivity rate exceeding 90% among bacteriologically confirmed cases evaluated for drug-resistant tuberculosis in Katsina State, Northwestern Nigeria. The similarity between these findings suggests that TB remains a major public health challenge in several regions of Nigeria despite ongoing control efforts. Much lower rates have however also been reported by Emorinken et al. (2023) with 41.3% prevalence among TB suspects attending a rural teaching hospital in Edo State, South-South Nigeria, 21.9% prevalence documented in a multi-state Nigerian screening study by (Odume et al., 2024) a large systematic TB screening project across six Nigerian states and that of 13.25% prevalence reported by Ajide et al. (2020) among suspected pulmonary TB patients in North-Central Nigeria.

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A Nigerian prison-based active screening study also reported a TB detection yield of about 7% prevalence among presumptive inmates, showing much lower prevalence in broader screening contexts compared to highly selected hospital populations (Adepoju et al., 2025). The observed difference may be attributable to variations in study population, geographical setting, sample size, diagnostic methods, and the epidemiological characteristics of the study areas. Nevertheless, both studies underscore the continued public health significance of TB in the region. This finding reflects the continued public health challenge posed by TB in Nigeria, which remains among the countries with the highest TB burden globally (World Health Organization [WHO], 2023).

The predominance of TB among young adults observed in this study is consistent with global and regional trends, where TB disproportionately affects individuals within their most economically productive age group, thereby contributing to significant socio-economic consequences (WHO, 2023; Dodd et al., 2021).

In this study, the high rate of positivity (70.7%) was observed with the Ziehl-Neelsen microscopy method. Such a similar trend has been noted in findings from high-burden diagnostic settings in Nigeria and similar African contexts, where ZN microscopy has demonstrated sensitivity ranging from 50% to about 80% depending on bacillary load and patient selection (Ejeh et al., 2018; Arora & Dhanashree, 2020; Demelash et al., 2023) and a Nigerian diagnostic evaluation reported that Ziehl–Neelsen microscopy achieved 80% sensitivity, showing strong detection capacity in a high-risk clinical population (Eze et al., 2024). These findings suggest that although ZN microscopy is less sensitive than molecular methods, it can yield high detection rates in high-risk or high-prevalence clinical populations. Okon et al. (2017) also reported that ZN microscopy demonstrated 61.1% sensitivity, showing improved detection in high-risk populations. Ziehl-Neelsen (ZN) microscopy in this study demonstrated a high positivity rate, reaffirming its utility as a rapid and cost-effective screening tool, particularly in resource-limited settings. This is consistent with the recent evidence from Mesfin et al. (2022) which further supports the findings of this study, demonstrating that ZN microscopy achieved satisfactory positivity rates among patients with pulmonary tuberculosis. This is particularly in settings where bacterial loads were high. Also a study by Nikpor et al. (2023) which reported that Ziehl–Neelsen staining remains widely utilized in low-income countries due to its low operational cost, ease of implementation, and relatively good performance in patients with high bacillary loads. The authors emphasized that despite the introduction of molecular diagnostic techniques, smear microscopy continues to serve as the primary diagnostic approach in many developing nations.

Culture on Lowenstein-Jensen (LJ) medium, although associated with a longer turnaround time and a higher risk of contamination, remains the reference standard for TB diagnosis. Its importance lies in its high sensitivity and its capacity for comprehensive drug susceptibility testing, which is essential for guiding treatment in cases of drug-resistant TB (Forbes et al., 2018; WHO, 2023). This study reports a relatively high contamination rate highlighting the importance of strict adherence to laboratory biosafety procedures and effective sample decontamination protocols to ensure reliable results. This is consistent with the study by Pfyffer and Wittwer (2021), which reported that contamination rates above acceptable laboratory thresholds are often associated with inadequate specimen handling, improper decontamination procedures, poor aseptic techniques, and delays in specimen processing. Their findings demonstrated that effective digestion decontamination procedures are critical for minimizing contamination while preserving viable mycobacteria for culture. Horne et al. (2022) also observed that strict implementation of laboratory quality management systems, routine biosafety monitoring, and staff training significantly reduced contamination rates in mycobacterial culture laboratories. They emphasized that maintaining contamination rates within recommended limits is necessary to ensure accurate TB diagnosis and surveillance. All of this is important because the high contamination rate resulted in a low detection rate (47.3%) using this method, significantly reducing the effectiveness of the technique.

The GeneXpert MTB Ultra assay demonstrated rapid detection of *Mycobacterium tuberculosis* underscoring its critical role in the early diagnosis of drug-resistant TB. This aligns with findings from more recent ev0aluations which confirm that GeneXpert provides high sensitivity and specificity.

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In addition, recent evidence reported by Lee et al. (2022) indicates that GeneXpert (Xpert MTB Ultra) contributes substantially to reducing diagnostic delays by 1–3 days in drug-sensitive TB. Compared with conventional culture-based drug susceptibility testing, which may require several weeks, GeneXpert can provide results within approximately two hours, facilitating timely clinical decision-making and infection control measures and providing similar levels of detection as seen in this study. Therefore, the findings of this current study align with recent research supporting the GeneXpert MTB Ultra assay as a highly effective tool for the rapid diagnosis of tuberculosis and early detection of rifampicin-resistant disease.

Overall, the findings of this study emphasize the complementary roles of molecular, microscopic, and culture-based diagnostic techniques. The integration of rapid molecular diagnostics such as GeneXpert with conventional culture methods offers a more comprehensive and reliable approach to TB diagnosis, particularly in high-burden settings like Nigeria. Strengthening such integrated diagnostic strategies is essential for improving case detection, reducing transmission, and enhancing TB control efforts.

Conclusion

This study highlights the high burden of tuberculosis in the South-South region of Nigeria and demonstrates that no single diagnostic method is sufficient for comprehensive TB detection though many facilities rely on passive TB case detection, often missing asymptomatic TB cases. Ziehl-Neelsen microscopy remains useful for rapid, low-cost screening in resource-limited settings but is limited by low sensitivity and inability to detect drug resistance. The GeneXpert MTB Ultra assay provides rapid and reliable detection of *Mycobacterium tuberculosis* and rifampicin resistance, supporting early diagnosis and treatment initiation. Culture on Lowenstein-Jensen medium, despite its longer turnaround time and risk of contamination, remains the gold standard for confirmatory diagnosis and drug susceptibility testing. Overall, an integrated diagnostic approach combining molecular, microscopic, and culture-based methods is essential for improving diagnostic accuracy and strengthening TB control in Rivers State and similar high-burden settings.

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